Republic of Korea, [6]Asia-Pacific Center for Theoretical Physics, Pohang, Republic of Korea, [7]KAIST Institute Korea Advanced Institute of Science and Technology (KAIST), the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea.
Inositol pyrophosphates such as IP_7 (5-diphosphoinositol pentakisphosphate) are highly energetic inositol metabolites containing pyrophosphate bonds. While IP_7 are known to regulate various biological events like metabolism, the molecular site of IP_7 action in vesicle trafficking remains elusive. Here, we showed that IP_7 potently inhibits synaptic vesicle exocytosis in both *invitro* reconstitution and cell culture settings. IP_7 was identified as a high-affinity ligand for synaptotagmin 1 (Syt1), a calcium sensor essential for membrane fusion. Interactions of IP_7 with Syt1 reduced the binding between Syt1 and Ca^{2+} as well as endowed Syt1 with its own negative effect, thereby suppressing Syt1 activation. These findings reveal a role of IP_7 as an inhibitor of the exocytotic pathway and expand our understanding of the signaling mechanisms of inositol pyrophosphates.

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C2B Domain in Synaptotagmin I Induces Membrane Bending Only After Conformational Change
Zhe Wu, Schulten Klaus.
University of Illinois Urbana Champaign, Urbana, IL, USA.
Neuronal exocytosis is mediated by a Ca^{2+}-triggered membrane fusion event that joins synaptic vesicles and presynaptic membrane. In this event, synaptotagmin I plays a key role as a Ca^{2+} sensor protein that binds to and bends the presynaptic membrane with its C2B domain and, thereby, triggers membrane fusion. We report free energy calculations according to which C2B-induced membrane bending is preceded by a Ca^{2+}- and membrane-dependent conformational transition, in which C2B attaches to the membrane, moves its C-terminal helix from the orientation seen in the membrane-free crystal/NMR structures as pointing away from the membrane (helix up), to an orientation pointing towards the membrane (helix down). In the C2B ''helix down'' state, lipid tails in the proximal membrane bilayer leaflet interact with the moved helix and become disordered, while tails in the distal leaflet, to keep in contact with the proximal leaflet, become stretched and ordered. The difference in lipid tail packing between the two leaflets results in an imbalance of pressure across the membrane and, thereby, causes membrane bending. The lipid disordering in the proximal membrane leaflet should facilitate Ca^{2+}-triggered membrane fusion.

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Mitofusin Proteins Tether Proteoliposomes as Shown by Cryo-EM
Jeanne Morin-Leisk, Jenny Hinshaw.
National Institutes of Health, Bethesda, MD, USA.
A balance between mitochondrial membrane fusion and fission is required for normal mitochondrial morphology and function. Shifts in mitochondrial morphology as a result of changes in the balance of membrane fusion and fission accompany cellular differentiation and changes in metabolic state. It is thought that the severe, early-onset peripheral neuropathy Charcot-Marie-Tooth type 2A disease is a manifestation of mitochondrial fusion malfunction as a result of a mutation of one of the two human mitofusins, mitofusin 2. Our aim is to reveal the mechanism by which mitofusins cause membrane fusion. Both mammalian mitofusins contain a large GTPase domain at their N-terminus followed by two transmembrane domains and a short C-terminal domain. It is predicted that GTP hydrolysis and mitofusin conformational changes are coupled to membrane fusion. However, the precise mechanism for mitofusin-mediated membrane fusion remains an open question. In fact, it is still unknown if mitochondrial outer membrane fusion proceeds through the canonical steps of tethering, docking, fusion, and disassembly. To gain insight into the conformational changes that lead to membrane fusion we are examining the structure of mitofusins in a lipid bilayer. As two-pass transmembrane proteins, mitofusins have proven difficult to express and purify in their full-length state and are poor candidates for crystallography. Here we present a purification strategy and the first cryo-em images of full-length mitofusins in a lipid bilayer. In addition, we show that mitofusins tether proteolipsosomes by forming oligomers that interact in trans between synthetic membranes. This implies that mitofusins are sufficient for driving the first step of membrane fusion.

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Role of Model Proteins on Membrane Fusion
Hemant K. Kashyap, Michael L. Klein, Giacomo Fiorin.
Institute for Computational Molecular Science, Temple University, Philadelphia, PA, USA.
Within neural cells, synaptic vesicles carry neurotransmitters (signaling molecule) through the plasma membrane. In order to do so synaptic vesicles must fuse with the plasma membrane, so that the enclosed neurotransmitter can cross it. Typically, without fusion proteins membrane fusion occurs on a very long time scale. However, it has been established that the fusion occur via fusion proteins (FPs), which initially are bound to one or both of the fusing membranes via a trans-membrane (TM) helix, utilize energetically favorable conformational transitions to lower the activation energy for membrane fusion, and thus are key participants in shaping the energy landscape by facilitating bilayer-bilayer apposition and dehydration as bilayers come into more intimate contact. It is also widely accepted that the trans-membrane (TM) part of the FPs has vital role in governing the fusion, fusion does not occur if TM is replaced with lipid molecules. In order to understand how the TM segment of FPs facilitates membrane fusion, we used a model protein that mimics mostly the TM part of the FPs, of similar dimensions to an α-helix. In doing so, the length of the model protein was chosen to match the thickness of a POPC bilayer. To retain the trans-membrane orientation of the model proteins without adding the soluble domains, we truncated the ends with polar groups. The middle portion was kept non-polar. We observed via coarse-grained molecular dynamics simulations that the self-aggregation of the model proteins greatly enhances the rate of formation of hemi-fused intermediate states. Also, the model-proteins if present in relatively higher concentration drive the attachment of a 20nm size vesicle to a flat bilayer.

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Waiting Times for Fusion Depend on the Number of Snares at the Fusion Site
Hakhamanesh Mostafavi[1], Benjamin S. Stratton[1], Jason M. Warner[1], Erdem Karatekin[2], Ben O'Shaughnessy[1].
[1]Chemical Engineering, Columbia University, New York, NY, USA, [2]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT, USA.
SNARE proteins mediate membrane fusion in fundamental cellular processes such as exocytosis. Formation of SNAREpin complexes, from vesicle associated v-SNAREs and target-membrane associated t-SNAREs, is thought to trigger fusion. However, the detailed mechanisms remain unclear and widely varying SNARE requirements for fusion are reported, 2-15 in vivo and 1-11 in vitro. Here we mathematically modeled the collective behavior of SNAREpins at the fusion site. The SNAREpins organize into a ring, as indicated by experiment, and steric interactions between SNAREpins expand the ring and pull membranes together, while the membranes repel one another through hydration and electrostatic forces. The net effect is a high pressure at the intermembrane contact point, sufficient to fuse the membranes should it exceed the measured pressure threshold for fusion [Wong et al., Biophys J, 1999]. Monte Carlo simulations showed that this pressure fluctuates considerably: with fewer SNAREpins, the mean pressure was lower, the probability of super-fusion pressure sharply reduced, and the waiting time for fusion increased. Thus, consistent with experiment, any number of SNAREpins can drive fusion, but the waiting time is strongly dependent on that number. For example, for a typical membrane composition, fast fusion of 50nm vesicles is predicted to require 11 SNAREpins, but 4 SNAREpins also achieve fusion ~50 times more slowly. We also find that waiting times increase for larger vesicles and for longer SNARE linker domains, in qualitative agreement with studies reporting lower fusion activity with elongated linkers. Our results suggest that fully zippered SNAREs work in concert to trigger fusion, by generating high local pressures that destabilize membranes into fusion pores. This naturally explains the widely reported variations in SNARE requirements for fusion.

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Field Theoretic Approach for the Energetics of Stalk Formation
Rolf Ryham[1], Thomas Klotz[1], Lihan Yao[1], Fredric S. Cohen[2].
[1]Mathematics, Fordham University, Bronx, NY, USA, [2]Molecular Biophysics and Physiology, Rush University, Chicago, IL, USA.
A numerical procedure was developed to calculate the minimum energy pathway for stalk formation between two flat and parallel lipid bilayer membranes. Bilayers were modeled as axially symmetric, freely deformable surfaces with lipid director fields. Energies were obtained by calculating the elastic energy–splay, tilt, and stretching–of each monolayer, along with undulation and hydration forces that repel membranes from each other. The topological transition between the planar and stalk phase was explicitly treated by allowing the bilayer to form a circular fissure along the axis of symmetry; the energy of fissure was approximated by the water-hydrocarbon surface and the area of exposed acyl chains. Generally, stalk formation is not spontaneous; it requires that external energy, such as provided by fusion proteins, be supplied to the bilayers. To quantify the energy required to surmount the